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Megalaria yunnanensis sp. nov. from Yunnan, China

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Abstract—*Megalaria yunnanensis* from southern China is described as a new species. The lichen is diagnosed by its bi-layered exciple, presence of atranorin, zeorin, and fumarprotocetraric acid in the thallus, ascospore size (20–25 × 5–7.5 µm), and the lack of indentations from the cell lumina into the center of the spore septum.

Key words —East Asia, *Lecanorales*, lichenized fungi, *Ramalinaceae*, taxonomy

Introduction

Megalaria (*Ramalinaceae*) was erected for the single species *M. grossa* (Pers. ex Nyl.) Hafellner (Hafellner 1984). The genus is recognized by its crustose thallus, biatorine apothecia, greenish to dark purple epihymenium, (2–)8-spored ascus, *Lecanora*-, *Bacidia*-, or *Biatora*-type asci, and 1-septate, ellipsoidal to subglobose, colourless, thick-walled ascospores (Ekman & Tønsberg 1996; Brodo & al. 2001: 428–429; Galloway 2004; Lendemer 2007; Sanderson 2009; Fryday & Lendemer 2010; McCarthy & Elix 2016; McMullin & Lendemer 2016).

Megalaria contains about 48 species worldwide (Lendemer & al. 2016, McMullin & Lendemer 2016, McCarthy & Elix 2016, Su & Ren 2017, Elix & McCarthy 2018). In China, only two species, *M. hainanensis* Q. Ren and *M. laureri* (Hepp ex Th. Fr.) Hafellner have been reported (Su & Ren 2017). During our study of the lichen flora of Mt. Wuliang in Jingdong county, Yunnan province, China, we collected a species of *Megalaria* new to science.

Materials & methods

The specimens were collected in Yunnan Province, China, and are preserved in Lichen Section of the Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU). The specimens were examined morphologically using a COIC XTL7045B2 stereo-microscope and anatomically with an Olympus CX41 polarizing microscope and photographed under Olympus SZX16 and BX61 with DP72. Thallus and medulla were tested with K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite) for identification. Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with system C (Orange & al. 2001).

Total genomic DNA was extracted with DNeasy Plant Mini Kit according to the manufacturer's instructions and purified with PCR quick-spin™ PCR Product Purification Kit. The ITS1-5.8S-ITS2 regions were amplified in a C1000TM automatic thermocycler using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990) in a 50 μL volume containing 5 units of 1 μl Taq DNA Polymerase (Sangon Biotech), 5 μL ITS buffer, 37.2μl ddH2O, 0.8 μL 10 μM F primer solution, 0.8 μL 10 μM R primer solution, 3.2 μL 2.5 mM per dNTP solution, and 2 μL genomic DNA. PCR thermal cycling parameters were initial denaturation at 98°C for 2 min, followed by 35 3-step cycles (98°C for 10 s, 54°C for 10 s, 72°C for 1 min), and a final 5 min extension at 72°C. PCR products were sanger-sequenced by Sangon Biotechnology Company.

All raw sequences were assembled and edited using SeqMan 7.0. The ITS data set comprised two newly generated sequences from the holotype and a paratype of our new species and eight relevant ITS sequences of *Megalaria* (plus two outgroup sequences) downloaded from GenBank. The sequences were aligned in MAFFT version 7 (Katoh & al. 2005) with the parameters set to default values. Ambiguous regions were excluded using Gblocks (Talavera & Castresana 2007) with default settings. There were 479 positions total in the final dataset. Maximum likelihood (ML) was used to reconstruct the phylogenetic tree (Fig. 2). *Biatora subduplex* (Nyl.) Printzen and *B. meiocarpa* (Nyl.) Arnold were used as outgroup (McMullin & Lendemer 2016). All characters were equally weighted and treated as unordered.

Taxonomy

Megalaria yunnanensis C.X. Wang & L. Hu, **sp. nov.** Fig. 1

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Differs from *Megalaria albocincta* by its longer ascospores and its lack of indentations from the cell lumina into the center of the spore septum.

Type: China. Yunnan province, Jingdong county, Mt. Wuliang, 24°24′02″N 100°45′44″E, alt. 2200 m, on bark, 7 Aug. 2017, R. Tang 20170963 (**Holotype**, SDNU; GenBank MK348528).

Etymology: The specific epithet *yunnanensis* refers to Yunnan province, where this species was found.

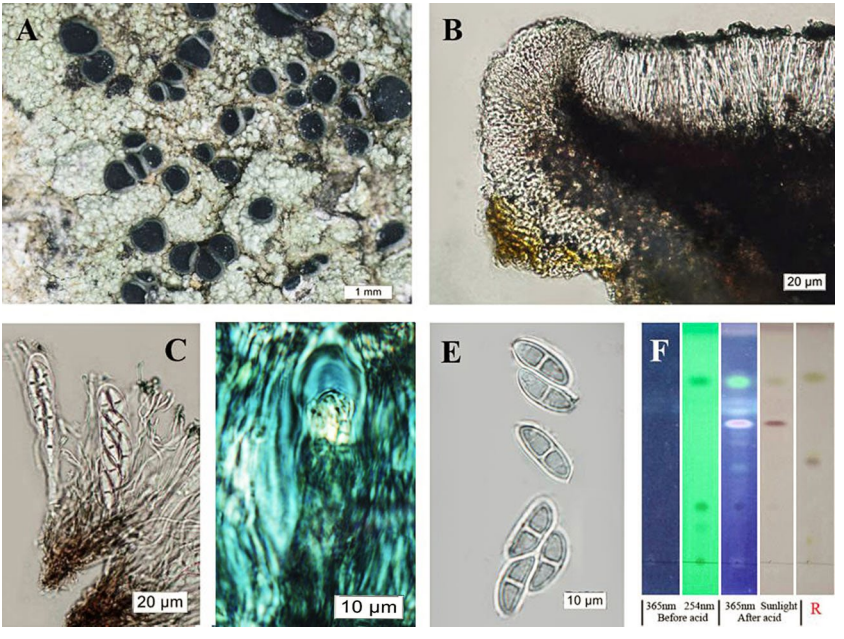

Fig. 1. *Megalaria yunnanensis*. (Holotype, SDNU (Tang 20170963)). A. Thallus and apothecia; B. Apothecial section; C. Asci and ascospores; D. Amyloid reaction of ascus, *Biatora*-type; E. Ascospores; F. TLC chromatograms (R = *Lethariella cladonioides*).

Thallus crustose, verrucose protuberance, corticolous, white, without soredia. Apothecia biatorine, adnate, circular in outline, 0.38–1 mm diam., margins pale, waxy white, becoming excluded with age; discs black, flat, not pruinose, occasionally with few crystals. Epihymenium dark green to black, 22.5–52.5 μm tall, K–, N+ red; hymenium hyaline, 50–67.5 μm tall, K–, N–; subhymenium colourless to red-brown or black, 37.5–50 μm tall, K+ red, N+ red-brown to red; hypothecium black, 25–65 μm tall, K–, N+ red; paraphysis hyaline, simple, easily free, I+ blue. Asci 8-spored, 45–62.5 × 11.25–15 μm, *Biatora*-type. Ascospores ellipsoid, colourless, thick walled, 1-septate, not halonate, 20–25 × 5–7.5 μm (n = 32). Pycnidia not seen.

Chemistry—Cortex and medulla K+ yellow, C–, KC–; thallus UV–; atranorin, zeorin, and fumarprotocetraric acid detected by TLC.

Additional specimens examined: **CHINA**. **Yunnan**, **Jingdong**, Mt. Wuliang, 24°24′02″N 100°45′44″E, alt. 2200 m, on bark, 7 Aug. 2017, R. Tang 20170805,

GenBank MK348527; 20170806; 20170820; 20170838; 20170856; 20170870; 20170896; 20170904; 20170918; 20170923; 20170926; 20170956; 20170962; 20170966 (SDNU).

Distribution—The type specimen was collected from Mt. Wuliang, Jingdong, Yunnan, which has a subtropical monsoon climate. The average annual temperature, relative humidity, and rainfall are 18.3°C, 77%, and 108.7 cm. This species grows on bark and is known only from the type locality.

Discussion

Megalaria yunnanensis is similar to *M. albocincta* (Degel.) Tønsberg, *M. alligatorensis* Lendemer, *M. anaglyptica* (Kremp.) Fryday & Lendemer, and *M. pulverea* (Borrer) Hafellner & E. Schreiner, all of which are characterized by a bi-layered exciple with a prosoplectenchymatous outer layer and an inner layer composed of a textura intricata with large intercellular spaces and a thalline chemistry of atranorin, zeorin, and fumarprotocetraric acid. However, the other four species are separated from *M. yunnanensis* by their shorter or smaller ascospores—*M. albocincta:* 13–17 × 6.5–8.5 µm, *M. alligatorensis:* 12–14 × 3.8–5.5 µm, *M. anaglyptica:* 17–22 × 4–6 µm, and *M. pulverea:* 10–16(–19) × 4.5–6.5 µm (Ekman & Tønsberg 1996, Kalb 2007, Sanderson 2009, Lendemer & al. 2016).

Furthermore, *M. albocincta* has two-sided indentations from the cell lumina into the center of the spore septum (Ekman & Tønsberg 1996); *M. alligatorensis* has a *Bacidia*-type ascus and greenish-blue thallus that forms small circular patches (Lendemer & al. 2016); *M. anaglyptica* has a thick granular thallus and dirty greyish brown subhymenium (Kalb 2007); and *M. pulverea* has a sorediate granular thallus and bigger apothecia (0.5–2.2 mm diam.; Sanderson 2009).

As shown in Fig. 2, *M. yunnanensis* is sister to *M. pulverea*, but the genetic distance (Kimura 2-parameter model) between these two species is quite large (0.198–0.207). Interestingly, a sterile sorediate species *M. alleniae* Lendemer & McMullin from southeastern North America (McMullin & Lendemer 2016) is chemically similar to *M. yunnanensis*, but our new species obviously differs from *M. alleniae* morphologically and molecularly (with a 0.212 genetic distance separating *M. alleniae* and *M. yunnanensis*).

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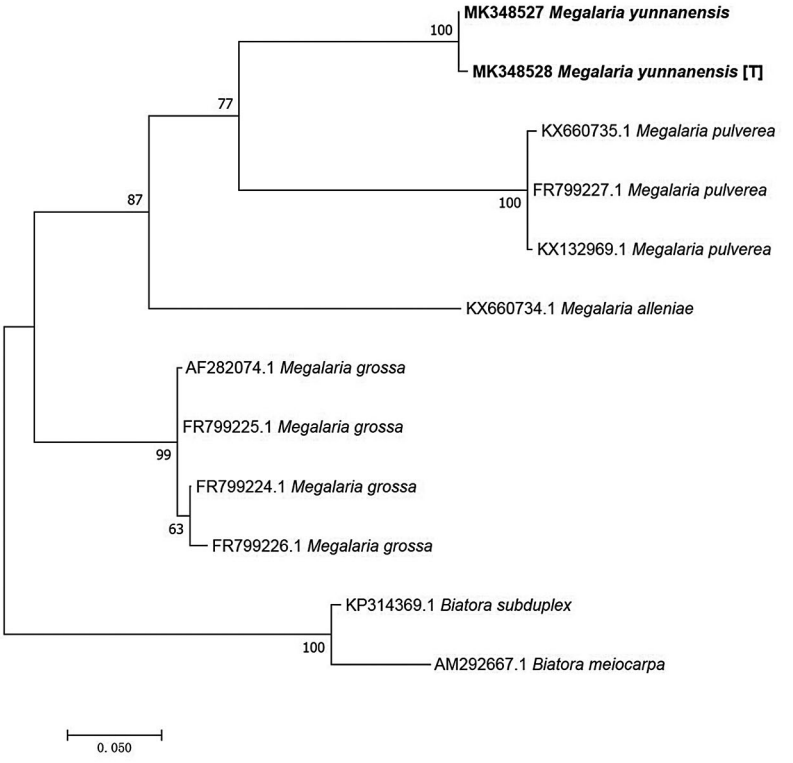

Fig. 2. Molecular phylogenetic tree illustrating the position of *Megalaria yunnanensis* in *Megalaria* s.l. based on ITS sequences. *Biatora* species were selected as the outgroup. Maximum likelihood bootstrap support values >50% are shown on nodes.

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